J. Braz. Chem. Soc., Vol. 20, No. 5, 826-831, 2009.
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0103 - 5053 $6.00+0.00

Article

Design, Synthesis and Anticonvulsant Activity Evaluation of 7-Substituted-4*H*-[1,2,4] triazino[3,4-*a*]phthalazin-4-one Derivatives

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Neste estudo, uma nova série de derivados de 4*H*-[1,2,4]triazina[3,4-*a*]ftalazin-4-ona substituídos em C-7 foi sintetizada como potenciais agentes anticonvulsivantes. Suas atividades anticonvulsivantes foram avaliadas pelo teste do eletrochoque máximo (MES), e suas neurotoxicidades, pelo teste "rotarod" de neurotoxicidade. Os resultados farmacológicos mostraram que a 7-hexiloxi-4*H*-[1,2,4]triazina[3,4-*a*]ftalazin-4-ona, **4e**, foi a mais potente, com dose efetiva (ED_{50}) de 6,6 mg kg^{-1} e dose tóxica mediana (TD_{50}) de 39,4 mg kg^{-1}, proporcionando um índice de proteção ($PI=TD_{50}/ED_{50}$) de 6,0.

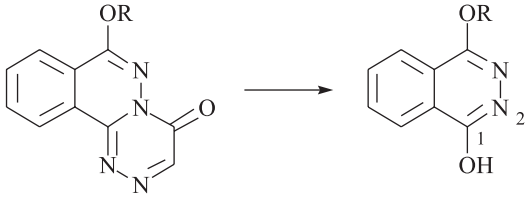
In this study, a novel series of 7-substituted-4*H*-[1,2,4]triazino[3,4-*a*]phthalazin-4-one derivatives was synthesized as potential anticonvulsant agents. Their anticonvulsant activities were evaluated by the maximal electroshock (MES) test, and their neurotoxicities were evaluated by the rotarod neurotoxicity test. The pharmacological results showed that 7-hexyloxy-4*H*-[1,2,4] triazino[3,4-*a*]phthalazin-4-one **4e** was the most potent with median effective dose (ED_{50}) value of 6.6 mg kg^{-1}, median toxicity dose (TD_{50}) of 39.4 mg kg^{-1}, providing a protective index ($PI=TD_{50}/ED_{50}$) value of 6.0.

Keywords: triazino, phthalazine, anticonvulsant, neurotoxicity

Introduction

Epilepsy, a ubiquitous disease characterized by recurrent seizures, afflicts more than 60 million people worldwide according to epidemiological studies.[1] For epilepsy treatment, nearly 95% of clinically available drugs were approved before 1985 and they could provide satisfactory seizure control for 60-70% of patients. These drugs, however, also cause notable adverse side effects such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity and megaloblastic anemia,[2-4] and even life threatening conditions.[5] Research to find more effective and safer antiepileptic drugs is, therefore, imperative and challenging in medicinal chemistry.

As part of our program directed toward the search for central nervous system (CNS) agents, the synthesis and evaluation of anticonvulsant activities of 7-substituted-4*H*-[1,2,4]triazino[3,4-*a*]phthalazin-4-one derivatives is herein reported. Some phthalazine derivatives (**I**, Figure 1) were reported to display anticonvulsant activity.[6] In an attempt to discover a novel anticonvulsant compound with better activity, we introduced a triazino ring at the first and second positions of the 4-alkoxyphthalazin-1-ol (**I**, Figure 1). The new compounds were evaluated as anticonvulsant agents in experimental epilepsy models. The rotarod assay was performed in mice to evaluate the neurotoxicity of the compounds. The anti-maximal electroshock (anti-MES) activity and the neurotoxicity of the marketed agent carbamazepine were evaluated in our laboratory under the same conditions for the purpose of comparison.



4a-4s **I**

Figure 1. Structure of compound **I** and target compounds.

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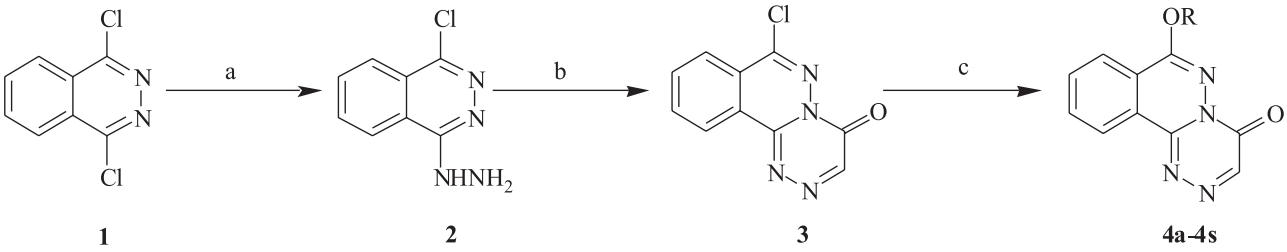


Regents and conditions: a) $NH_2NH_2 \cdot H_2O/C_2H_5OH$, 80 °C, 1h; b) $HCOCOOH/C_2H_5OH$, r. t., 2h; DMF, reflux, 3h; c) alkanol/DMF, NaOH.

Scheme 1. Synthesis of compounds **4a**-**4s.**

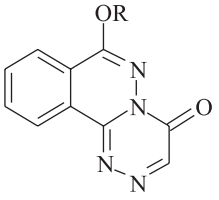
Results and Discussion

The target compounds **4a**-**4s** were synthesized according to Scheme 1. Compound **1**, a key substrate for the whole reaction, was prepared by an established procedure.[7] The starting material phthalic anhydride reacted with hydrazine hydrate in ethanol to yield 2,3-dihydrophthalazine-1,4-dione, which reacted further with the refluxing $POCl_3$ to yield compound **1**. Compound **1** reacted further with hydrazine hydrate in ethanol to afford compound **2** (1-hydrazine-4-chrorophthalazin). Briefly, to a solution of hydrazine hydrate in ethanol, a solution of compound **1** in ethanol was added dropwise at room temperature, and the mixture was stirred at 80 °C for 1 h. Then, half of the solvent was removed under reduced pressure, and the mixture was poured into petroleum ether. The precipitate was filtered and washed with petroleum ether, and then kept below 0 °C. The compounds obtained were pure enough for the next step. Compound **2** reacted further with glyoxylic acid monohydrate in ethanol at room temperature, then evaporated the solvent under reduced pressure, the residue continued to react in refluxing DMF for about 2h to obtain target compound **3**.[8] The target compounds **4a**-**4i** and **4j**-**4s** were prepared from compound **3** reacting with an appropriate amount of alcohol and phenol, respectively, in a solution of NaOH in DMF.

The initial evaluation (phase I) of the anticonvulsant activity of synthesized compounds **4a**-**4s** (Table 1) indicated that all compounds displayed anticonvulsant activity at 100 mg kg^{-1}, while compounds **4d**, **4f**, **4j**, **4n** and **4o** were effective at 30 mg kg^{-1}. On the basis of phase I screening, the compounds which exhibited anti-MES activity at 30 mg kg^{-1} (4 effective in 5 tested mice), were then subjected to phase II trials for quantification of their anticonvulsant activity and neurotoxicity in mice. The data were also compared with the marketed anticonvulsant drug carbamazepine, the results were shown in Table 2.

Analyzing the activities of 19 triazino[3,4-*a*]phthalazin-4-one derivatives **4a**-**4s**, the following structure-activity relationship was obtained. The length of the alkyl chain appeared to have a direct impact on anticonvulsant activity of the 7-alkoxy-4H-[1,2,4]triazino[3,4-a]phthalazin-

Table 1. Primary evaluation of compounds **4a**-**4s** in anticonvulsant activity (test drug administered i.p.)[a]



Compound	R	MES test[b]	
		30[c]	100
4a	$-C_2H_5$	1/5[d]	5/5
4b	*i*-C_3H_7	3/5	5/5
4c	*n*-C_4H_9	3/5	5/5
4d	*n*-C_5H_{11}	5/5	—[e]
4e	*n*-C_6H_{13}	5/5	—
4f	*n*-C_7H_{15}	4/5	—
4g	*n*-C_8H_{17}	4/5	—
4h	*n*-C_9H_{19}	4/5	—
4i	*n*-$C_{10}H_{21}$	0/5	4/5
4j	$-C_6H_5$	5/5	—
4k	$-C_6H_4$(*p*-CH_3)	3/5	5/5
4l	$-C_6H_4$(*o*-CH_3)	2/5	5/5
4m	$-C_6H_4$(*m*-CH_3)	1/5	5/5
4n	$-C_6H_4$(*p*-F)	5/5	—
4o	$-C_6H_4$(*p*-Cl)	5/5	—
4p	$-C_6H_4$(*p*-Br)	1/5	5/5
4q	$-C_6H_4$(*p*-OCH_3)	3/5	5/5
4r	$-C_6H_4$(*o*-OCH_3)	1/5	5/5
4s	$-C_6H_4$(*p*-NH_2)	2/5	5/5

[a]All of the tested compounds were dissolved in Polyethylene glycol-400. [b]The maximal electroshock test was carried out 15 min after administration of the test compounds. [c]Doses are denoted in milligrams *per* kilogram. [d]The effective number in 5 tested mice. [e]not tested.

Table 2. Phase II quantitative anticonvulsant data in mice (test drug administered i.p.)

Compound	ED_{50} [a]	TD_{50} [b]	PI[c]
4d	12.1(8.9-16.5)[d]	39.4(28.9-53.7)	3.3
4e	6.6(4.8-9.0)	39.4(28.9-53.7)	6.0
4f	19.0(13.9-25.9)	38.0(27.9-51.8)	2.0
4g	22.8(16.7-31.1)	47.3(34.7-64.5)	2.1
4h	27.4(20.1-37.3)	54.8(40.2-74.7)	2.0
4j	19.7(14.5-26.8)	45.6(33.5-62.1)	2.3
4n	13.2(9.7-18.0)	40.9(29.4-56.7)	3.1
4o	11.0(7.9-15.2)	39.4(28.9-53.7)	3.6
carbamazepine	6.6(4.8-9.4)	45.6(33.5-62.1)	6.9

[a]ED_{50}-median effective dose required to assure anticonvulsant protection in 50% animals. [b]TD_{50}-median toxic dose eliciting minimal neurological toxicity in 50% animals. [c]PI protective index (TD_{50}/ED_{50}). [d]95% confidence limits given in parentheses.

4-one derivatives. From compound **4a** to **4e**, as alkyl chain length increased, anticonvulsant activity gradually increased with the compound **4e** (with the *n*-hexyloxy substituted group) being the most active. The trend reversed, however, when the alkyl chain had more than six carbon atoms. From compound **4f** to **4h**, as alkyl chain length increased, ED_{50} gradually decreased from 19.0 to 27.4 mg kg^{-1}. Compound **4e**, with ED_{50} value of 6.6 mg kg^{-1}, was comparable to reference agent carbamazepine in anti-MES activity, but it exhibited neurotoxicity with TD_{50} value of 39.4 mg kg^{-1}, so obtained a smaller PI value of 6.0 than that of carbamazepine (PI=6.9). As shown in Table 1, the anticonvulsant activity decreased obviously when alkyl chain number lengthened to 10, compound **4i** was ineffective at dose of 30 mg kg^{-1}.

The pharmacological results of three methyl-group-substituted derivatives revealed that the position of the substituted groups on the phenyl ring greatly influenced the anticonvulsant activity; the *p*-CH_3 derivative **4k** exhibited higher anticonvulsant activity than the *o*-CH_3 derivative **4l** and *m*-CH_3 derivative **4m**. In these halogen derivatives, the Cl atom had a larger contribution to the anticonvulsant activity than the F and Br atom. In the phase II screening, the 4-Cl derivative **4o** exhibited stronger anticonvulsant activity with an ED_{50} value of 11.0 mg kg^{-1} than the 4-F derivative **4n** with an ED_{50} value of 13.2 mg kg^{-1} in the MES test.

Compared with non-substituted phenoxy compound **4j**, the introduction of electron-donor group such as methyl, methoxy and amino did not increase the anticonvulsant activity, although all these compounds displayed anticonvulsant activity at 100 mg kg^{-1}.

Conclusions

In summary, all synthesized compounds displayed anticonvulsant activity at 100 mg kg^{-1} and their structure-activity relationship was discussed in the present study. In particular, we found that 7-hexyloxy-4*H*-[1,2,4] triazino[3,4-*a*]phthalazin-4-one (**4e**) possessed the most potential anticonvulsant activity with an ED_{50} value of 6.6 mg kg^{-1}, which was comparable to reference agent carbamazepine in anti-MES activity.

Experimental

Chemistry

Melting points were determined in open capillary tubes and were uncorrected. ^{1}H-NMR spectra were measured on an AV-300 (Bruker, Switzerland), and all chemical shifts were given in parts *per* million relative to tetramethysilane.

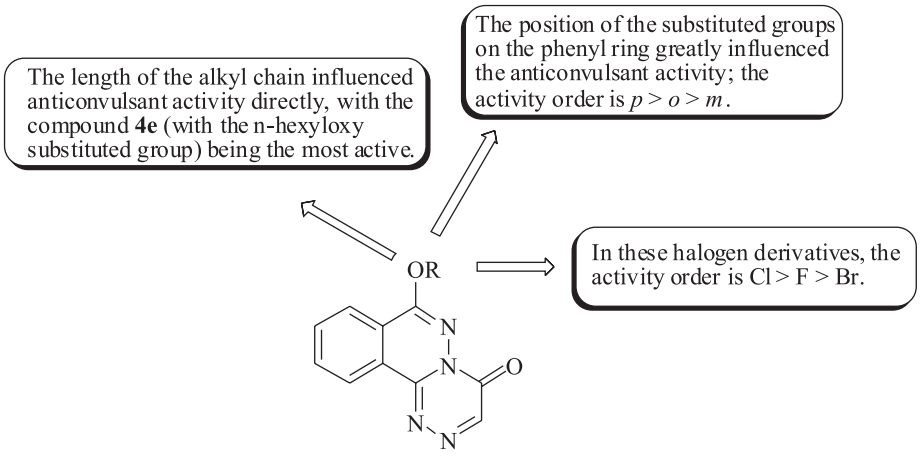


Figure 2. The structure and anticonvulsant activity relationship summary of title compounds.

Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). Elemental analyses were performed on a 204Q CHN (PerkineElmer, USA). Microanalyses of C, N, and H were performed using a Heraeus CHN Rapid Analyzer. The major chemicals were purchased from Aldrich Chemical Corporation. All other chemicals were of analytical grade.

*Synthesis of 1-hydrazino-4-chrorophthalazin (**2**)*

To a solution of hydrazine hydrate (6.28 g, 125.6 mmol) in 20 mL of ethanol, a solution of compound **1** (5 g, 25.1 mmol) in 60 mL ethanol was added dropwise at room temperature. The mixture was stirred and heated at 80 °C for 1 h, then, half of the solvent was removed under reduced pressure, and the solution was poured into petroleum ether. The precipitate was filtered and washed with petroleum ether, and then kept below 0 °C. The resulting crude intermediates were used for the next step.

*Synthesis of 7-chloro-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one (**3**)*

A solution of compound **2** (4 mmol) and glyoxylic acid monohydrate (4 mmol) in 30 mL of ethanol was stirred for 2 h at room temperature to obtain the acid intermediate. The mixture was evaporated under reduced pressure. The acid intermediate was then reacted in refluxing DMF for 3 h. The solution was evaporated to dryness, and the oily residue was filtered on a silica gel chromatographic column (ethyl acetate) to give a white or light yellow solid. Yield 48%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.90-8.76 (m, 4H, Ar-H), 9.01 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1708 (C=O). MS *m/z* 233 (M+1), 235 (M+3). *Anal. Calc.* for $C_{10}H_5ClN_4O$: C 51.63, H 2.17, N 24.08. Found: C 51.70, H 2.18, N 23.99.

*The general procedure for the synthesis of 7-substituted-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one derivatives (**4a**-**4s**)*

Compound **3** (0.4 g, 1.72 mmol) and appropriate alkanol or substituted phenol (1.72 mmol) were added to a solution of sodium hydroxide (1.72 mmol) in DMF with stirring and refluxing for about 3 h. After the solvent was removed under reduced pressure, the solid residue was purified by silica gel chromatography with dichloromethane: methanol (20:1). The yield, melting point, spectral data of each compound is given below.

*7-Ethoxy-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one (**4a**)*

mp 162-164 °C, yield 84%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.58 (t, 3H, *J* 7.08 Hz, –CH_3), 4.54-4.61 (q, 2H, *J* 7.05 Hz, –OCH_2–), 7.77-8.64 (m, 4H, Ar-H), 8.84 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1714 (C=O). MS *m/z* 243 (M+1). *Anal. Calc*. for $C_{12}H_{10}N_4O_2$: C 59.50, H 4.16, N 23.13. Found: C 59.68, H 4.27, N 22.98.

*7-Isopropoxy-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one (**4b**)*

mp 92-94 °C, yield 85%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.51 (d, 6H, *J* 6.15 Hz, –$CH(CH_3)_2$), 5.41-5.45 (m, 1H, *J* 6.15 Hz, –OCH–), 7.74-8.61 (m, 4H, Ar-H), 8.82 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1716 (C=O). MS *m/z* 257 (M+1). *Anal. Calc*. for $C_{13}H_{12}N_4O_2$: C 60.93, H 4.72, N 21.86. Found: C 61.09, H 4.74, N 21.70.

*7-Butoxy-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one (**4c**)*

mp 101-103 °C, yield 81%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.06 (t, 3H, *J* 7.29 Hz, –CH_3), 1.94 (m, 2H, –CH_2–), 4.52 (t, 2H, *J* 6.48 Hz, –OCH_2–), 7.80-8.69 (m, 4H, Ar-H), 8.84 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1710 (C=O). MS *m/z* 271 (M+1). *Anal. Calc*. for $C_{14}H_{14}N_4O_2$: C 62.21, H 5.22, N 20.73. Found: C 62.37, H 5.34, N 20.57.

*7-Pentoxy-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one (**4d**)*

mp 119-121 °C, yield 84%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.99 (t, 3H, *J* 6.99 Hz, –CH_3), 1.47-1.52 (m, 4H, –CH_2CH_2–), 1.93-1.98 (m, 2H, –CH_2–), 4.50 (t, 2H, *J* 6.48 Hz, –OCH_2–), 7.79-8.65 (m, 4H, Ar-H), 8.85 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1716 (C=O). MS *m/z* 285 (M+1). *Anal. Calc*. for $C_{15}H_{16}N_4O_2$: C 63.37, H 5.67, N 19.71. Found: C 63.50, H 5.79, N 19.51.

*7-Hexoxy-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one (**4e**)*

mp 102-104 °C, yield 88%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, 3H, *J* 7.00 Hz, –CH_3), 1.27-1.41 (m, 6H, –$(CH_2)_3$–), 1.92-1.97 (m, 2H, –CH_2–), 4.50 (t, 2H, *J* 6.48 Hz, –OCH_2–), 7.79-8.65 (m, 4H, Ar-H), 8.84 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1715 (C=O). MS *m/z* 299 (M+1). *Anal. Calc*. for $C_{16}H_{18}N_4O_2$: C 64.41, H 6.08, N 18.78. Found: C 64.54, H 6.10, N 18.60.

*7-Heptoxy-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one (**4f**)*

mp 85-87 °C, yield 83%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.90 (t, 3H, *J* 6.81 Hz, –CH_3), 1.34-1.55 (m, 8H, –$(CH_2)_4$–), 1.92-1.97 (m, 2H, –CH_2–), 4.50 (t, 2H, *J* 6.48 Hz, –OCH_2–), 7.76-8.64 (m, 4H, Ar-H), 8.83 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1707(C=O). MS *m/z* 313 (M+1). *Anal. Calc*. for $C_{17}H_{20}N_4O_2$: C 65.37, H 6.45, N 17.94. Found: C 65.57, H 6.49, N 17.73.

7-Octoxy-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one ***(4g)***

mp 83-85 °C, yield 80%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, 3H, *J* 6.81 Hz, $-CH_3$), 1.27-1.55 (m, 10H, $-(CH_2)_5-$), 1.92-1.97 (m, 2H, $-CH_2-$), 4.50 (t, 2H, *J* 6.48 Hz, $-OCH_2-$), 7.79-8.67 (m, 4H, Ar-H), 8.83 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1713 (C=O). MS *m/z* 327 (M+1). *Anal. Calc.* for $C_{18}H_{22}N_4O_2$: C 66.24, H 6.79, N 17.17. Found: C 66.38, H 6.85, N 17.01.

7-Nonoxy-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one ***(4h)***

mp 78-80 °C, yield 86%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.86 (t, 3H, *J* 6.91 Hz, $-CH_3$), 1.17-1.51 (m, 12H, $-(CH_2)_6-$), 1.92-1.97 (m, 2H, $-CH_2-$), 4.53 (t, 2H, *J* 6.50 Hz, $-OCH_2-$), 7.77-8.67 (m, 4H, Ar-H), 8.84 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1718 (C=O). MS *m/z* 341 (M+1). *Anal. Calc.* for $C_{19}H_{24}N_4O_2$: C 67.04, H 7.11, N 16.46. Found: 67.24, H 7.15, N 16.25.

7-Decoxy-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one ***(4i)***

mp 72-74 °C, yield 87%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.86 (t, 3H, *J* 6.91 Hz, $-CH_3$), 1.15-1.51 (m, 14H, $-(CH_2)_7-$), 1.91-1.97 (m, 2H, $-CH_2-$), 4.50 (t, 2H, *J* 6.48 Hz, $-OCH_2-$), 7.77-8.67 (m, 4H, Ar-H), 8.83 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1719 (C=O). MS *m/z* 355 (M+1). *Anal. Calc.* for $C_{20}H_{26}N_4O_2$: C 67.77, H 7.39, N 15.81. Found: C 67.91, H 7.48, N 15.58.

7-Phenoxy-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one ***(4j)***

mp 150-152 °C, yield 79%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.30-8.72 (m, 9H, Ar-H), 8.74 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1709 (C=O). MS *m/z* 291 (M+1). *Anal. Calc.* for $C_{16}H_{10}N_4O_2$: C 66.20, H 3.47, N 19.30. Found: C 66.34, H 3.49, N 19.10.

7-(4-Methphenoxy)-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one ***(4k)***

mp 158-160 °C, yield 84%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.45 (s, 3H, $-CH_3$), 7.18-8.72 (m, 8H, Ar-H), 8.75 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1711 (C=O). MS *m/z* 305 (M+1). *Anal. Calc.* for $C_{17}H_{12}N_4O_2$: C 67.10, H 3.97, N 18.41. Found: C 67.25, H 4.03, N 18.19.

7-(2-Methyphenoxy)-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one ***(4l)***

mp 154-156 °C, yield 87%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.26 (s, 3H, $-CH_3$), 7.21-8.71 (m, 8H, Ar-H), 8.73 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1713 (C=O). MS *m/z* 305 (M+1). *Anal. Calc.* for $C_{17}H_{12}N_4O_2$: C 67.10, H 3.97, N 18.41. Found: C 67.24, H 4.03, N 18.21.

7-(3-Methylphenoxy)-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one ***(4m)***

mp 135-137 °C, yield 80%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.45 (s, 3H, $-CH_3$), 7.13-8.73 (m, 8H, Ar-H), 8.76 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1709 (C=O). MS *m/z* 305 (M+1). *Anal. Calc.* for $C_{17}H_{12}N_4O_2$: C 67.10, H 3.97, N 18.41. Found: C 67.23, H 3,99, N 18.22.

7-(4-Fluorophenoxy)-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one ***(4n)***

mp 151-153 °C, yield 85%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.17-8.72 (m, 8H, Ar-H), 8.74 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1722 (C=O). MS *m/z* 309 (M+1). *Anal. Calc.* for $C_{16}H_9FN_4O_2$: C 62.34, H 2.94 N 18.17. Found: C 62.49, H 2.98 N 18.01.

7-(4-Chlorophenoxy)-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one ***(4o)***

mp 145-147 °C, yield 84%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.25-8.71 (m, 8H, Ar-H), 8.75 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1715 (C=O). MS *m/z* 325 (M+1), 327 (M+3). *Anal. Calc.* for $C_{16}H_9ClN_4O_2$: C 59.18, H 2.79, N 17.25. Found: C 59.32, H 2.85, N 17.04.

7-(4-Brominephenoxy)-4H-[1,2,4]triazino[3,4-a] phthalazin-4-one ***(4p)***

mp 159-161 °C, yield 80%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.20-8.72 (m, 8H, Ar-H), 8.76 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1710 (C=O). MS *m/z* 370 (M+1). *Anal. Calc.* for $C_{16}H_9BrN_4O_2$: C 52.05, H 2.46, N 15.18. Found: C 52.29, H 2.50, N 14.94.

7-(4-Methoxyphenoxy)-4H-[1,2,4]triazino[3,4-a] phthalazin-4-one ***(4q)***

mp 165-167 °C, yield 85%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.88 (s, 3H, $-OCH_3$), 7.00-8.70 (m, 8H, Ar-H), 8.75 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1706 (C=O). MS *m/z* 321 (M+1). *Anal. Calc.* for $C_{17}H_{12}N_4O_3$: C 63.75, H 3.78, N 17.49. Found: C 63.92, H 3.85, N 17.21.

7-(2-Methoxyphenoxy)-4H-[1,2,4]triazino[3,4-a] phthalazin-4-one ***(4r)***

mp 216-218 °C, yield 79%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.78 (s, 3H, $-OCH_3$), 7.06-8.69 (m, 8H, Ar-H), 8.72 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1716 (C=O). MS *m/z* 321 (M+1). *Anal. Calc.* for $C_{17}H_{12}N_4O_3$: C 63.75, H 3.78, N 17.49. Found: C 63.94, H 3.84, N 17.19.

*7-(4-Aminophenoxy)-4H-[1,2,4]triazino[3,4-a]phthalazin-4-one (**4s**)*

mp 181-183 °C, yield 86%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.31 (s, 2H, $-NH_2$), 6.77-8.69 (m, 8H, Ar-H), 8.75 (s, 1H, 3-H). IR (KBr) ν/cm^{-1}: 1720 (C=O). MS *m/z* 321 (M+1). *Anal*. *Calc*. for $C_{16}H_{11}N_5O_2$: C 62.95, H 3.63, N 22.94. Found: 63.23, H 3.69, N 22.57.

Pharmacology

The MES test and rotarod test were carried out according to procedures described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health (USA).[9,10] All compounds, which were dissolved in polyethyleneglycol-400, were evaluated for anticonvulsant activities with Kunming mice in the 18-25 g weight range. Groups of 10 mice were given a range of intraperitoneal doses of the test drug until at least three points were established in the range of 10-90% seizure protection or minimal observed neurotoxicity. From the plots of these data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slopes of the regression line and the standard error of the slopes were calculated by means of a computer program written by the National Institute of Neurological Disorders and Stroke.

MES test

Seizures were elicited with a 60 Hz alternating current of 50 mA intensity in mice. The current was applied *via* corneal electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. The derivatives in MES test were evaluated 15 min after the administration of the compounds.

Rotarod test[11]

Fifteen minutes after the administration of the compounds (with different doses), the animals were tested on a 1-in. diameter; knurled plastic rod rotating at 6 rpm for 1 min. Neurotoxicity was indicated by the inability of an animal to maintain equilibrium in each of three trials.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 30760290, 30860340) and Important Item Foundation of Ministry of Education P.R. China (No. 20070422029).

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Received: August 29, 2008
Web Release Date: March 13, 2009